REMARKS

I. Status Summary

Claims 1, 6, and 8 are pending in the present U.S. patent application and have been examined by the United States Patent and Trademark Office (hereinafter "the Patent Office").

Claims 1, 6, and 8 have been rejected under 35 U.S.C. § 103(a) upon the contention that the claims are unpatentable over Cereijido *et al.* (1993) Suppl 17 *J Cell Science* 127-132 (hereinafter "Cereijido") in view of Grunicke *et al.* (1996) 36 *Advan Enzyme Regul* 385-407 (hereinafter "Grunicke").

Claims 4-6 have been canceled without prejudice. Applicants respectfully reserve the right to file one or more continuation and/or divisional applications with claims directed to the subject matter of the canceled claims.

Claim 1 has been amended. Support for the amendment can be found throughout the specification as filed, including particularly at page 9, line 25. Thus, no new matter has been added by the amendment to claim 1.

New claims 28-30 have been added. Support for the new claims can be found throughout the specification as filed, including particularly on page 8, lines 5-7 (absorption site is intestinal epithelium) and at page 22, lines 3-5 (oral or parenteral administration). Thus, no new matter has been added to the application as a result of the inclusion of the new claims.

Reconsideration of the application based on the application as amended and in light of the remarks set forth below is respectfully requested.

II. Response to the Obviousness Rejection

Claims 1, 6, and 8 have been rejected under 35 U.S.C. § 103(a), upon the contention that the claims are unpatentable over Cereijido in view of Grunicke. According to the Patent Office, Cereijido teaches that inhibition of phospholipase C (PLC) reduces the development of the transepithelial electrical resistance (TER; also abbreviated as TEER), which the Patent Office asserts is a measure for the paracellular transport through cells, and activation of PLC increases TER. Thus, the Patent Office contends that one of ordinary skill in the art would have understood *a priori* that PLC

plays an important role in paracellular transport across the intestinal epithelium and that upon administration of a PLC inhibitor the paracellular membrane permeability would be enhanced (*see* Official Action at page 4). The Patent Office further asserts that Grunicke teaches that hexadecylphosphocholine (HePC, Miltefosine, n = 15) is an inhibitor of PI-specific PLC.

After careful consideration of the rejection and the Patent Office's basis therefor, applicants respectfully traverse the rejection and submit the following remarks.

The Patent Office asserts that Cereijido discloses that inhibition of PLC by neomycin reduces the development of TER. Applicants respectfully submit that this assertion is based on what is believed to be an inaccurate interpretation of the data presented in Cereijido.

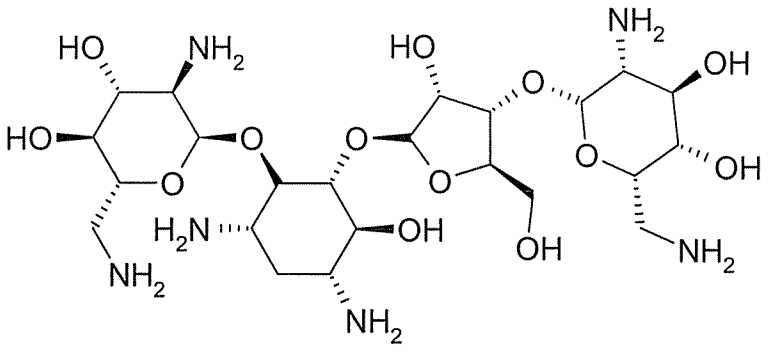
Applicants have submitted herewith a DECLARATION OF DHIREN R. THAKKER, PH.D. PURSUANT TO 37 C.F.R. §1.132 (hereinafter the "Thakker Declaration"). As set forth in the Thakker Declaration, Cereijido is believed to contain many errors, and the model of junction formation presented is based on a flawed interpretation of the data described therein.

For example, Cereijido is asserted to show that in MDCK cells, formation of tight junctions (as indicated by an increase in TER) caused by increasing the Ca^{2+} concentration from 5 μM to 1.2 mM is blocked by neomycin (*see* Figure 4). The authors purport to suggest that the blocking of the tight junction formation by neomycin is due to its PLC inhibitory activity. Thus, they conclude that PLC is involved in the formation of tight junctions. They propose a mechanism of formation of tight junctions (Figure 5) that involves activation of PLC by cell-cell contact, leading to the release of Ca^{2+} from intracellular storage and activation of PKC, ultimately causing the junctional complex proteins to transfer from a storage vesicle to the cell membrane and formation of the tight junction.

Applicants respectfully submit that there are several aspects of the model proposed by Cereijido that raise serious questions about the authors' conclusion that neomycin blocked the formation of tight junction by inhibition of PLC. These aspects are discussed in the Thakker Declaration at Items 5-7.

First, the authors provide no evidence that neomycin treatment of MDCK cells causes inhibition of cellular PLC. In fact, it was known as of the filing date of the instant application that neomycin has many actions on cells, and as such is not a selective PLC inhibitor. Item 5 of the Thakker Declaration cites Polascik *et al.*, 1987 and Sipma *et al.*, 1996 (true and accurate copies of which are provided as **Exhibits B** and **C**, respectively), which applicants respectfully submit describe neomycin as alternatively a PLC activator (*see* Polascik Abstract and page 818, right column, first full paragraph). Applicants further respectfully submit that Sipma discloses that neomycin can block histamine-induced Ca^{2+} entry independent of PLC activation, which led the authors to conclude that "neomycin is not a suitable tool to study the effects of messengers generated downstream of phospholipase C activation on receptor mediated and capacitive Ca^{2+} entry in DDT 1 MF-2 cells" (*see* Sipma at page 211, left column, first full paragraph). The Thakker Declaration indicates that Polascik and Sipma are but two examples of many publications that are believed to show that neomycin is not a selective inhibitor of PLC and further that its cellular and pharmacological effects are due to multiple mechanisms, none of which can definitively be attributed to PLC inhibition.

In Item 6, the Thakker Declaration states that as a member of the aminoglycoside class of antibiotics, neomycin would not be expected to cross cell membranes to modulate PLC activity. As indicated in Item 6 of the Thakker Declaration, neomycin has the following structural formula:



Neomycin

It is believed that its chemical structure would be understood by those of skill in the art to prevent it from crossing cell membranes and entering cells. Particularly, and as set forth in Item 7 of the Thakker Declaration, neomycin is characterized by over 15 H-bonding sites, several cationic functionalities, and large molecular weight, which would prevent it from crossing cell membranes and entering cells.

From this and in view of his expertise in the area of drug transport mechanisms, Dr. Thakker asserts that it would be highly unlikely that applying neomycin to MDCK cells as described in Cereijido would lead to inhibition of intracellular PLC (*see* Thakker Declaration at Item 7). Thus, and in view of the fact that Polascik *et al.*, 1987 and Sipma *et al.*, 1996 were published after Cereijido and before the filing date of the instant application, applicants respectfully submit that as of the filing date of the instant application, one of ordinary skill in the art would have understood that the data presented in Cereijido would not have suggested to one of ordinary skill in the art that neomycin's activity in preventing TJ formation resulted from PLC inhibition.

Turning now to the assertion in Cereijido that thyrotropin-1 releasing hormone (TRH) promotes TJ formation, applicants respectfully submit that here as well the authors have misinterpreted the data presented in the reference. The Patent Office's attention is directed to Items 8-10 of the Thakker Declaration. With respect to Item 8 of the Thakker Declaration, it is stated that Cereijido provides no evidence that any effect on TER by TRH is mediated by PLC activation. According to the Thakker Declaration, the data in Figure 4 of Cereijido suggests that TRH treatment of MDCK cells enhances TER by approximately 2-fold over that produced by 1.2 mM Ca^{2+}. From this, the authors appeared to conclude that TRH activates PLC through TRH receptors, and further that the activated PLC increases TER over that produced by 1.2 mM Ca^{2+} by stimulating the formation of tight junctions beyond those formed by 1.2 mM Ca^{2+} alone.

Item 9 of the Thakker Declaration points out how these assertions by the authors of Cereijido are also erroneous. Specifically, the Thakker Declaration states that it was known prior to the filing date of the instant application that MDCK cells do not express TRH receptors. As set forth in Yeaman *et al.*, 1996, a true and accurate copy of which is provided as **Exhibit D**, MDCK cells do not express TRH receptors endogenously (*see* page C755, left column, last paragraph; *see also* Figure 2A of Yeaman). Since Yeaman

was published after Cereijido, applicants respectfully submit that one of ordinary skill in the art would have understood that TRH could not stimulate PLC in MDCK cells because the cells lack TRH receptors. As such, it is believed that the effect of TRH on increasing TER in MDCK cells reported by Cereijido could not have been due to activation of PLC.

Furthermore, as set forth in Item 10 of the Thakker Declaration, no evidence is presented in Cereijido to show that PLC was in fact activated by TRH treatment of MDCK cells. Like neomycin, applicants respectfully submit that TRH is known to affect multiple cellular mechanisms (*see* Barros *et al.*, 1998, a true and accurate copy of which is attached as **Exhibit E**).

Thus, applicants respectfully submit that as of the filing date of the instant application, one of ordinary skill in the art would have understood that the conclusions reached in Cereijido were inaccurate, and as such, this reference would not have provided one of ordinary skill in the art with a reasonable expectation that paracellular permeability at an absorption site could be enhanced by administering a phospholipase C inhibitor to a subject.

However, even assuming *arguendo* that Cereijido suggested that tight junction formation could be influenced by PLC modulators, applicants respectfully submit that the Patent Office has not appreciated the difference between tight junction formation as described in Cereijido and the situation *in vivo* at an absorption site. As an initial matter, applicants respectfully traverse the Patent Office's assertion that applicants' arguments with respect to this issue is improper as being directed to a feature that is not recited in the claims (*i.e.*, tight junctions already formed). Applicants respectfully submit that one of ordinary skill in the art would clearly understand that instant claim 1 relates to enhancing paracellular permeability at an absorption site in a subject through the administering of the effective amount of the phospholipase C inhibitor.

Applicants further respectfully submit that one of ordinary skill in the art would also understand that the absorption sites in a subject have pre-formed tight junctions. As such, it is believed that there is no basis for the Patent Office to assert that this feature would not be understood to be implicit in the claims, and further that the Patent

Office's assertion that this amounts to reading a limitation from the specification into the claims is also believed to be in error.

Nonetheless, in an effort to facilitate prosecution, applicants have amended claim 1 to recite *inter alia* that the absorption site is a site where tight junctions are presented. Support for the amendment can be found throughout the specification as filed, including particularly at page 8, line 25. Thus, no new matter has been added by the amendment to claim 1.

Continuing with the instant rejection, the Patent Office has asserted that "membranes are constantly in flux, therefore the inhibition of additional tight junctions will indeed increase the paracellular permeability any time the phospholipase C inhibitor is administered" (*see* Final Official Action at page 4). Applicants respectfully submit that the Patent Office has provided no support for this assertion, and thus has not met its burden under 35 U.S.C. § 103.

To elaborate, applicants respectfully submit that according to M.P.E.P. § 2144.03,

> Official notice without documentary evidence to support an examiner's conclusion is permissible only in some circumstances. While "official notice" may be relied on, these circumstances should be rare when an application is under final rejection or action under 37 CFR 1.113. Official notice unsupported by documentary evidence should only be taken by the examiner where the facts asserted to be well-known, or to be common knowledge in the art are capable of instant and unquestionable demonstration as being well-known. As noted by the court in *In re Ahlert*, 424 F.2d 1088, 1091, 165 USPQ 418, 420 (CCPA 1970), the notice of facts beyond the record which may be taken by the examiner must be "capable of such instant and unquestionable demonstration as to defy dispute" (citing *In re Knapp Monarch Co.*, 296 F.2d 230, 132 USPQ 6 (CCPA 1961)).

M.P.E.P. § 2144.03 (emphases added). Applicants respectfully submit that the Patent Office's assertions with respect to tight junction formation at absorption sites falls far short of the requirement of "instant and unquestionable demonstration as to defy dispute". Thus, the Patent Office has not met its burden in asserting without documentary evidence that new tight junction formation is occurring at an appreciable rate in established membranes.

Furthermore, applicants respectfully submit that the processes involved in *de novo* formation of tight junctions as described in Cereijido are not the same as the processes involved in modifications of pre-formed tight junctions as are present at absorption sites *in vivo*. Further, the effect of a mediator (such as PLC) on the formation of tight junctions is not the same as its effect the mediator would be expected to have on pre-formed tight junctions.

Additionally and as set forth in Item 12 of the Thakker Declaration, it is believed that the Patent Office's apparent assumption that if PLC affects the formation of tight junctions it would also be expected to affect permeability of pre-formed tight junctions is believed to be in error. Item 12 of the Thakker Declaration continues by analyzing the effect of PLC in promoting fusion of vesicles containing tight junction component proteins with a cell membrane, as proposed in Figure 5 of Cereijido. According to the Thakker Declaration, Cereijido suggests that tight junction formation occurs by phosphorylation of intracellular vesicles containing tight junction proteins.

In contrast, Item 13 of the Thakker Declaration indicates that the opening and closing of existing tight junctions (*i.e.*, tight junctions at an absorption site membrane) occurs in discrete substructures of the tight junctions (e.g., pores located therein). This is believed to not require complete dismantling and reassembly of the tight junctions. Thus, even if PLC were involved in vesicle fusion, this process is believed not to occur in pre-formed junctions, and thus the Cereijido model is inapplicable to tight junction modulation at absorption sites.

Particularly, and as set forth in Items 14-19 of the Thakker Declaration, it is believed that the permeability of pre-formed tight junctions can be modulated by altering the conformation of one or more tight junction proteins, and further that any mechanism involved in the formation of new tight junctions would not teach one of ordinary skill in the art how to modulate the permeability of pre-formed tight junctions.

Additionally, Item 20 of the Thakker Declaration addresses the Patent Office's apparent assertion that since the intestinal epithelium is continuously creating new tight junctions, an administered PLC inhibitor would be expected to impact formation of at least one tight junction and thus impact paracellular permeability. To summarize Item 20 of the Thakker Declaration, the location in which new intestinal epithelium cells are

formed – the crypts – are physically separated from the intestinal epithelium *per se*. Specifically, Dr. Thakker points out that new tight junctions are formed just as the cells are moving out of the crypts in a location that is physically separated from the vilus, the latter of which is the site of drug absorption in the intestinal epithelium. Dr. Thakker continues in Item 20 as follows:

> As such, when the cells reach near the tip of the vilus and become available for interactions with drug molecules and their absorption, they have long since formed all of their tight junctions. Therefore, since the formation of new tight junctions occurs in the crypt and not in the vilus where drug absorption occurs, even if PLC inhibitors could inhibit tight junction formation, it is believed that a PLC inhibitor (e.g., a alkylphosphocholine) administered to a subject (i.e., in vivo) would not come in contact with cells at a time that they were forming a new tight junction due to the physical separation of the cells in the crypt from the cells on the vilus (i.e., the cells where absorption takes place).

Summarily, applicants respectfully submit that Cereijido does not provide a reasonable expectation that PLC inhibitors could be employed to enhance paracellular permeability in a subject. Applicants further respectfully submit that Grunicke does not cure this defect. Thus, applicants respectfully submit that the combination of Cereijido and Grunicke does not support the instant rejection of claim 1. Claims 6 and 8 depend from claim 1, and thus are also believed to be distinguished over the combination of Cereijido and Grunicke. Claim 6 has been canceled without prejudice. As a result, applicants respectfully submit that claims 1 and 8 are in condition for allowance, and respectfully solicit a Notice of Allowance to that effect.

III. Discussion of the New Claims

New claims 28-30 have been added. Support for the new claims can be found throughout the specification as filed, including particularly on page 8, lines 5-7 (absorption site is intestinal epithelium) and at page 22, lines 3-5 (oral or parenteral administration). Thus, no new matter has been added to the application as a result of the inclusion of the new claims.

Applicants respectfully submit that new claims 28-30 are believed to be distinguished over the cited combination of Cereijido and Grunicke for the reasons set

forth hereinabove with respect to pending claims 1, 6, and 8. Particularly, applicants respectfully submit that the combination of Cereijido and Grunicke fails to teach or suggest using the claimed alkylphosphocholines for increasing paracellular permeability *in vivo*, and thus cannot be interpreted to suggest the subject matter of claims 1, 6, 8, or 28-30.

Accordingly, applicants respectfully submit that claims 1, 8, and 28-30 are in condition for allowance, and respectfully solicit a Notice of Allowance to that effect.

CONCLUSION

In light of the above amendments and remarks, it is respectfully submitted that the present application is now in proper condition for allowance, and an early notice to such effect is earnestly solicited.

If any small matter should remain outstanding after the Patent Examiner has had an opportunity to review the above Remarks, the Patent Examiner is respectfully requested to telephone the undersigned patent attorney in order to resolve these matters and avoid the issuance of another Official Action.

DEPOSIT ACCOUNT

Although no fee is believed to be due with respect to the filing of the instant paper, the Commissioner is hereby authorized to charge any underpayment of fees, and to credit any over payment, to Deposit Account Number **50-0426**.

Respectfully submitted,
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